STSM report of Yasen Mutafchiev

Short Term Scientific Missions (STSM): "A multinationational field parasitological expedition on the track to the vector of *Onchocerca lupi"*
Host institution: Universidade de Èvora, Portugal; person in charge: Helder Cortes heldercortes@gmail.com
Period: 14/04/2014 to 19/04/2014
Applicant: Yasen Mutafchiev, PhD
Reference code: COST-ONLINE STSM-TD1303-17271

Approved amount: EUR 800

PURPOSE OF THE STSM

Short Term Scientific Missions was implemented according the provided working plan. STSM was aimed to strengthening the collaboration and networking between scientists from other participating COST countries. The visit to the University of Evora provided opportunities to work on neglected parasite *O. lupi* and to conduct the research on its possible vector.

This mission allowed participants to exchange experience in field and laboratory research methods and techniques not available in their own country and to acquire additional knowledge on specific environmental and epidemiological conditions concerning parasite and vectors.

The performed research activities were related to the TD1303 COST Action objectives of "One Health" concept in the ecology of vector-borne diseases (WG1) and investigating rare and emerging vector-borne pathogens (WG5).

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

1. Collecting and identification of haematophagus insects

Haematophagus insects were captured by dry ice baited traps or obtained by collected mature pupae of blackflies in the highly productive breeding site within the affected area was. Insects of three families of medical importance and potential vectors were collected: mosquitoes (Culicidae), blackflies (Simuliidae) and sand flies (Psychodidae: Phlebotominae).

2. Feeding the insects on the infected host

Collected individuals from traps (mosquitoes, blackflies and sand flies) were fed on the infected dog placed in the closed tent (3m x 3 m x 2 m). The feeding of insects was conducted by:

a) Releasing of insects (mosquitoes and blackflies) inside the tent, where the dog was continually exposed to bites.

Releasing of the insects collected by traps was performed in the evening hours. Recollecting of fed individuals, recognized by enlarged abdomen was conducted regularly each day in the morning hours by application of the insect aspirator. Unfed individuals were left in the tent until the next recollection. After the end of the experiment all of the visually unfed mosquitoes and blackflies were collect and preserved in ethanol 70% for further analysis.

b) Application of glass tubes, with 5-7 blackflies per tube for stimulated feeding of blackflies on the dog on the selected body region.
The tubes with females were placed and hold tight on skin of the forehead or inter-scapular region of the dog, where highest number of *O. lupi* microfilaria was previously detected.

3. Maintaining of the successfully fed insects

All of the fed individuals, mosquitoes and blackflies, were placed in plastic insect cages, supplied by the source for additional feeding (gauze soaked with 10% sugar solution) and covered by wet dark towel in order to maintain adequate humidity of about 80% and semi dark conditions to inhibit the excessive activity that would lead to energy overconsumption and affect the lifespan. All specimens that died before the feeding on the dog were morphologically identified to species level and stored in individual eppendorf tubes, containing 70% ethanol for further molecular analyses on the presence of helminths DNA.

The period of duration of the STSM was not long enough to observe the final results of the research, the development of microfilaria to the infective L_3 stage. In fact, at the moment when this report was wrote many of insects fed on the dog were still alive. Therefore, the last phases of the research started within the STSM action is still in process. Soon after the death of each fed insect individual, they are identified, dissected in the saline solution in order to check visually the presence of infective third stage larva of *O. lupi* and then placed in 70% ethanol for further molecular analysis on parasite detection.

4. Procedures on dogs

The applicants were trained by other STSM participants, experts in veterinary medicine and parasitologists, on the sampling procedures of diagnose canine filarioids in dogs:

a) How to collect blood and skin samples from dogs, after owners consent, to assess the presence of microfilariae of *Onchocerca lupi*, *Dirofilaria immitis*, *Dirofilaria repens, Acanthocheilonema reconditum* and *Cercopithifilaria* bainaespp. Skin samples were collected using individual 3mm diameter biopsy punches, performed on the head and inter-scapular region a dog.
b) Laboratory procedures of detection of microfilariae in blood smears and screening of skin snip samples for presence of skin-dwelling microfilariae.

DESCRIPTION OF THE MAIN RESULTS OBTAINED AND OUTCOMES

Majority of the collected blood sucking insects during the STSM were mosquitoes and blackflies. In the mosquito fauna the dominant species was Culex Pipiens Complex followed by *Ochlerotatus caspius*. Few specimens of *Oc. detritus* were also recorded. Blackfly fauna was represented by three species: *Simulium*

pseudequinum as the dominant species, *Simulium intermedium* and *S. velutinum*. All of the blackfly species identified during this mission have been recorded in Portugal in the past and listed in the last edition of the revised taxonomic and geographical inventory of world blackflies (Adler & Crosskey, 2014).

Ten dogs were sampled for microfilariae and all isolated species of microfilaria were morphologically and morphometrically identified. In particular, microfilariae of the following filarioid species infesting dogs were detected: *O. lupi*; *Cercopithifilaria bainae* and *Cercopithifilaria* sp. II sensu Otranto et al. 2012.

The detection of *O. lupi* developing larvae is currently in still progress in Laboratory of Medical Entomology, University of Novi Sad (Serbia) where the applicant is affiliated.

Exchange of experience

During the STSM, the applicants introduced their experience in different fields of parasitology. I have been introduced in the methods of collecting of blood and skin snip samples from dogs and collecting and maintaining of haematophagous insects. From my side, as a parasitologist working on taxonomy of nematodes I introduced to the other applicants my experience on detecting and identification of microfilariae in blood smear and skin tissue of dogs, and methods of preparing of permanent microscopic slides with microfilariae.

Future collaboration

The STSM was very useful for the participants to establish long term collaborative interaction. Further joint projects on pathogens and their impact on important wild and domestic animals were discussed between me and the research team of the University of Evora (host institution), as well as, with the team headed by prof. Domenico Otranto (University of Bary).

Foreseen publications/articles resulting from the STSM (if applicable)

The results of this STSM will be published in 1 or 2 articles in peer-reviewed journals.

CONFIRMATION BY THE HOST INSTITUTION OF THE SUCCESSFUL EXECUTION OF THE STSM

The confirmation by the Host Institution will be given by Prof. Helder Cortes.

OTHER

I am very grateful to the COST Action TD1303 for the provided grant. I am obliged to Prof. Helder Cortes for the excellent organization in the course of the field studies and for making available facilities of the University of Evora.

Report done on May, 14th, 2014

Applicant and STSM participant:

Yasen Mutafchiev